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Female athlete health: The silent risks of high performance

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Abstract

Despite female athlete health becoming a growing area of interest in sport science research, there are still gaps to be filled in the literature. Low energy availability (LEA) and iron deficiency are two prevalent issues in women (or female athletes) that are under high loads of exercise stress from training. Thus, it is critical that measurement and identification of these parameters is accurate. The first part of this thesis reviews the literature surrounding LEA and iron deficiency with a focus on current identification techniques used in research and clinical settings. The second part of this thesis includes two original investigations. The first study, Chapter 2, had two aims; 1) to investigate the test-retest reliability of an indirect calorimetry analyser (Parvo Medics TrueOne), and 2) compare measured RMR with three RMR-predictive (pRMR) equations in female athletes. To address the first aim, 12 recreationally-exercising women (mean \pm SD; age 27.5 ± 12.3 y) performed two RMR assessments, on separate days, utilising the Parvo Medics TrueOne analyser. To address the second aim, 25 females (mean \pm SD; age 30.1 ± 10.2 y) underwent an RMR assessment using the Parvo Medics TrueOne analyser, which was compared to three calculated pRMR equations (Harris-Benedict [H-B], Mifflin-St Jeor [M], World Health Organisation [WHO]). Test-retest reliability for the TrueOne analyser was deemed acceptable (CV = 5.3%, ICC = 0.92). The validity of pRMR when compared to measured RMR showed low levels of agreement in all 3 predictive equations (M: CV = 21.4%, TEE = $269 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.16$, WHO: CV = 21.5%, TEE = $270 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.13$ H-B: CV = 21.6%, TEE = $270 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.13$).

The second study, Chapter 3, was designed to investigate specific health parameters related to iron, resting metabolic rate and energy balance in a cohort of highly-trained women. Thirteen highly-trained female endurance athletes (mean \pm SD; age 32 ± 7 y, training volume 18.5 ± 4.1 hrs) provided a blood test to assess iron markers (haemoglobin [Hb], haematocrit [Hct], C-reactive protein [Crp], serum iron, serum ferritin and transferrin). Additional measures included resting metabolic rate (RMR) assessment, dual-energy x-ray absorptiometry (DXA) scans, diet and training diaries and menstrual cycle tracking to identify any additional risk factors which may be associated with relative energy deficiency in sport. Analysis revealed that 46% (6/13) of this population had iron levels below optimal. Serum iron, serum ferritin and transferrin were all significantly decreased in the iron deficient (ID) group ($p < 0.05$). When compared to the non-iron deficient (non-ID) group haemoglobin (Hb) and haematocrit (Hct) were significantly lower in the ID group compared with the non-ID group ($p < 0.05$). The relationship between Crp and serum iron revealed a large negative correlation ($r = -0.66$), Crp and serum ferritin a very large negative correlation ($r = -0.72$) and transferrin presented a large correlation with Crp ($r = 0.70$). In conclusion, the outcomes of these studies demonstrate the need for greater stratification of risk thresholds for this (emerging) group of highly-trained, non-professional female athletes.

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Abbreviations

β -hCG - Beta-human chorionic gonadotropin

BMD – Bone mineral density

CI – Confidence interval

Crp – C-reactive protein

CV – Coefficient of variation

DXA – Dual-energy x-ray absorptiometry

EA – Energy availability

EI – Energy intake

FSH – Follicle stimulating hormone

H-B - Harris-Benedict

Hb – Haemoglobin

Hct – Haematocrit

ID – Iron deficiency

Kcal - Kilocalorie

LBM – Lean body mass

LEA – Low energy availability

LOA – Limits of Agreement

M – Mifflin-St Jeor

pRMR – Predicted resting metabolic rate

REDs – Relative energy deficiency in sport

RMR – Resting metabolic rate

SD – Standard deviation

sFer – Serum ferritin

TEE – Typical error of estimate

WHO – World health organisation

Thesis Overview

The format of this thesis includes four chapters; Chapter One contains a review of relevant literature surrounding female athlete health, specifically, resting metabolic rate (RMR) and iron deficiency, to introduce the reader to current practices and highlight any gaps in the literature. Chapter Two is an original investigation of RMR reliability and validity when compared to predictive equations. This chapter is presented in the style of an individual journal article as it has been submitted for review in the journal of *Performance Enhancement and Health*, therefore some information may be repeated throughout this thesis. Chapter Three investigates iron status, inflammation and bone health in a group of non-professional highly-trained female endurance athletes. Finally, Chapter Four, summarises the findings of both Chapter Two and Chapter Three and provides practical applications as well as suggested areas for further investigation in this line of research.

Chapter 1: Literature Review

Introduction

Female athletes are at a higher risk of clinical health issues than their male counterparts due to different physiological adaptations, dietary requirements and hormonal status (Ahmadi, Enayatizadeh, Akbarzadeh, Asadi, & Tabatabaee, 2010; McClung, Gaffney-Stomberg, & Lee, 2014; Warren & Perlroth, 2001). Female endurance athletes, in particular, face a unique set of challenges to maintain both optimal health and performance (Melin et al., 2015). Iron deficiency (ID) is reported to affect 60% of female athletes on varying scales of intensity (Cowell, Rosenbloom, Skinner, & Summers, 2003). While women are susceptible to suboptimal iron status due to negative iron balance contributed by inadequate dietary intake as well as losses through menstruation, female athletes may have a greater risk due to increased iron losses associated with hemolysis, sweating, gastrointestinal bleeding and exercise-induced acute inflammation (Auersperger et al., 2013; Ma, Patterson, Gieschen, & Bodary, 2013; Peeling, Dawson, Goodman, Landers, & Trinder, 2008). Often associated with female athlete health also is low energy availability (LEA). LEA is a condition that occurs when there is an imbalance between energy expenditure from exercise training and energy intake, is often an under-identified, but impactful health concern of female athletes. LEA is associated with a myriad of health issues such as menstrual dysfunction, compromised reproductive function, impaired bone health and thyroid function, and overall decreased athletic performance (De Souza et al., 2014; De Souza & Williams, 2004; Petkus, Murray-Kolb, & De Souza, 2017). These components are

collectively referred to Relative Energy Deficiency in Sport (REDs) (Mountjoy et al., 2014).

A subset of research is present in current literature in agreement with the deleterious effects female endurance athletes could (potentially) encounter if they develop an iron deficiency or fall into a state of LEA for extended periods (Mountjoy et al., 2014; Petkus et al., 2017; Slater, Brown, McLay-Cooke, & Black, 2017). While screening for risk factors of low iron, LEA and other symptoms of REDs are now starting to be implemented in high performance sport settings (Thein-Nissenbaum & Hammer, 2017), knowledge and awareness is still lacking in school-age through to non-professional elite athletes which puts them at a higher risk of long-term health issues and performance decreases (Hoch et al., 2009). This review aims to investigate current research underpinning iron deficiency and LEA in female athletes, with a focus on gaps in the literature that could provide better insight to prevention and screening techniques in female athletes below the professional threshold.

Section One: Iron Status in Female Athletes

Female endurance athletes are most at risk for low iron intake; an essential micronutrient in energy production pathways and a functional component of hemoglobin and myoglobin. Iron is necessary to aid in both physiological and cognitive functions of the body through the transportation of oxygen to active tissue (Beard, 2001). Additionally, iron plays an important role in the electron transport chain promoting oxidative phosphorylation, critical to general health

and athletic performance (Peeling et al., 2008; Rowland, 2012). Due to fluctuations across the menstrual cycle stored iron levels are somewhat variable in females, however, it is estimated to be around 2.5 g (Clenin et al., 2015). Of this stored iron, 1-2 g is generally lost during daily function (Nielsen & Nachtigall, 1998) with augmented iron losses shown in athletes (Shaskey & Green, 2000). These losses are recovered through dietary intake, via the consumption of heme, (found in meat products), and non-heme iron (occurring in vegetables and fortified cereals). The daily recommended iron intake for females is 18 mg/day (McClung et al., 2014). When iron stores are not recovered through adequate dietary intake, the body becomes depleted and if sustained, an iron deficiency may develop (Malczewska, Raczynski, & Stupnicki, 2000).

Identifying iron deficiency

It is known that increased prevalence of iron deficiency in female athletes is common (Table 1.), however discrepancies around what classifies iron deficiency still exist. Archer and Brugnara (2015) reference three stages of iron deficiency: a) 'storage iron depletion' where a drop in serum ferritin may be noticed however there are no significant haematological changes present; b) 'iron deficiency non-anaemic' where there may be an observed drop in haemoglobin (Hb) levels as well as other biochemical markers, however this could still be inside normal reference ranges and; c) 'iron deficiency anaemia' where multiple biochemical markers are outside normal reference ranges. While stage 3 (iron deficiency anaemia) is the most severe, all three stages of iron deficiency could have adverse effects on athletic performance (Clenin et al., 2015). Garvican and colleagues (2014),

investigated athletic performance and changes in iron deficiency in 27 highly-trained distance runners (with existing low iron stores, but not anaemia). Using intravenous iron supplementation for 6-weeks they noticed an increase in iron stores which may have also enhanced endurance capacity through erythropoiesis, and subsequently the oxygen carrying capacity of red blood cells.

Table 1. Prevalence of iron deficiency (ID) in physically active females.

Reference	Subjects	sFer cut off value used (ug/L)	Prevalence of ID
Alaunyte et al. (2014)	11 female distance runners (age: 32 ± 7 y)	<12	36%
Auersperger et al. (2013)	14 female runners. ID group (n = 7, age: 34.9 ± 4.7 y) & Non ID group (n=7, age: 31.4 ± 5.9 y)	<20	50%
Choe et al. (2001)	72 physically active females (age: 15-17 y)	<12	21%
Clement et al. (1987)	36 female winter Olympic athletes	<30	39%
Constantini et al. (2000)	25 female gymnasts (age: 13.5 ± 1.6 y)	<20	33%
DellaValle & Haas (2012)	48 female rowers grouped by iron status. ID (n = 24, age: 19.6 ± 1.1 y) & Non ID (n =24, age: 20.1 ± 1.1 y)	<20	27%
Di Santolo et al. (2008)	70 female athletes from various sports (age: 24.0 ± 4.22 y)	<12	27%
Gropper et al. (2006)	70 collegiate female athletes from various sports (age: 18-25 y)	<15	24%
Koehler et al. (2012)	97 female athletes of various sports (age: 16.3 ± 3.0 y)	<35	57%
Malczewska et al. (2001)	121 female athletes from various sports (age: 21.5 ± 3.4 y)	<20	26%
Milic et al. (2011)	359 female athletes of various sports (age: 20.40 ± 4.42 y)	<22	18%

Ostojic & Ahmetovic (2008)	15 female distance runners	<12	20%
Pate et al. (1993)	111 female runners (age: 29.9 ± 7.2 y)	<20	50%
Sandstrom et al. (2012)	57 female high school athletes of various sports (age: 16.8 ± 0.97 y)	<16	52%
Sinclair & Hinton (2005)	72 active females (age: 18-41 y)	<16	29%
Spodaryk et al. (1996)	40 female athletes from various sports	<20	20%
Woolf et al. (2009)	28 highly-active female (age: 20 ± 2 y)	<12	21%

* ID - Iron Deficient, sFer - Serum Ferritin

Serum ferritin (sFer) is a valid marker for determining iron deficiency (Jacobs & Worwood, 1975); however, variability in this marker exists in female athletes as inflammation and infection can cause serum ferritin levels to differ from iron storage status, consequently inferring a false negative diagnosis of ID (Van den Bosch et al., 2001). Other markers such as serum iron, serum transferrin, and transferrin may also be used in the identification of an iron deficiency (Archer & Brugnara, 2015). The sFer threshold indicative of an iron deficiency in the literature is inconsistent however and can range from <12 g/L to 35 g/L ferritin (Burden, Morton, Richards, Whyte, & Pedlar, 2015; Di Santolo et al., 2008; Milic et al., 2011; Pate et al., 1993; Peeling et al., 2007) creating uncertainty when comparing results. It has been suggested that athletes, especially endurance-based athletes, have increased losses leading to a higher prevalence of iron depletion (Nachtigall, Nielsen, Fischer, Engelhardt, & Gabbe, 1996; Sinclair & Hinton, 2005) indicating the need for a standardised threshold in athletes that differs to current clinical guidelines based on a sedentary population (Wians, 2018).

Effect of iron deficiency on athletic performance

It is well established that decreased iron status has an undesirable effect on general health. Research suggests an iron deficiency (with and without anemia) may compromise immune function, impair cognitive function and decrease metabolic efficiency as well as compromise overall bone health (Beard, 2001; DellaValle & Haas, 2011; Toxqui & Vaquero, 2015). Through the human performance lens, VO_{2max} (an individuals' maximal rate of aerobic energy capacity)

is fundamental to successful performance in endurance-oriented sports such as (mid-long distance) running, swimming, rowing and cycling (Costill, Thomason, & Roberts, 1973; Saltin & Astrand, 1967). As iron is an essential element to oxidative metabolism, diminished iron status can impair aerobic capacity, therefore reducing overall endurance performance (Hinton, Giordano, Brownlie, & Haas, 2000; Rowland, Deisroth, Green, & Kelleher, 1988).

Section Two: Low Energy Availability in Female Athletes

A growing number of female athletes, from recreational to elite, suffer from low energy availability (LEA) (Table 2.). LEA can be induced when energy intake is reduced, energy expenditure is increased, or (most commonly) a combination of the two (Loucks, Kiens, & Wright, 2011). LEA, not other factors associated with exercise, causes the development of exercise-induced amenorrhea, iron deficiency, and other physiological perturbations associated with REDs (Mountjoy et al., 2014).

Effects of LEA on health and wellbeing

Increased energy expenditure, combined with decreased energy intake can lead to adaptive neuroendocrine changes which result in the down regulation of many hormones that contribute to reproductive function (Lagowska & Kapczuk, 2016; Lagowska, Kapczuk, Friebe, & Bajerska, 2014; Melin et al., 2015; Muia, Wright, Onywera, & Kuria, 2016; VanHeest, Rodgers, Mahoney, & De Souza, 2014). Alongside this, it has also been observed that athletes showing signs of LEA have a lower RMR than those in energy balance (Melin et al., 2015). Original findings in

the literature deemed that hormonal balance is disrupted below the threshold of 30 kcal·kg/day⁻¹ relative to lean body mass/day⁻¹ (Loucks & Thuma, 2003), although there is evidence to suggest that below 45 kcal·kg/LBM/day⁻¹ is sub-optimal for normal function (Hoch et al., 2009; Melin et al., 2015). As little as 5-days below this threshold (>30 kcal·kg LBM/day⁻¹) has been shown to cause noticeable reductions in hypothalamic-pituitary-axis hormones, disrupting reproductive function and increasing levels of circulating cortisol (Loucks & Thuma, 2003).

Table 2. Prevalence of low energy availability (LEA) in physically active females.

11	Reference	Subjects	Prevalence of LEA	Comments
	Day et al. (2015)	25 Division 1 female runners (age: 19.5 ± 1.8 y)	53%	This study acknowledges optimal EA to be <45 kcal·kg/LBM/day ⁻¹ . 92% of subjects were below this. Average EA = 30.7 kcal·kg/LBM/day ⁻¹
	De Souza et al. (1998)	24 female exercisers (mean age: 27.8 y)	100%	When compared to a sedentary cohort EA was significantly lower in the exercising population ($p < 0.05$)
	Doyle-Lucas et al. (2010)	15 professional female ballet dancers (mean age: 24.3 y)	100%	When compared to a sedentary cohort EA was significantly lower in the dancers ($p < 0.01$)
	Koehler et al. (2013)	185 female athletes from various sports (age: 16.3 ± 2.8 y)	50%	Didn't take into account below optimal levels of EA in this study
	Lagowska & Kapczuk (2016)	31 female athletes (age: 18.1 ± 2.6 y) & 21 female ballet dancers (age: 17.1 ± 0.9 y)	100% of the ballet dancers, unclear in female athlete subjects.	EA was low in both study populations with athletes presenting EA levels of 28.3 ± 9.2 kcal·kg/LBM/day ⁻¹ and ballet dancers slightly lower with 21.7 ± 7.2 kcal·kg/LBM/day ⁻¹
	Melin et al. (2015)	25 female endurance athletes (age: 26.6 ± 5.6 y)	12%	44% were below optimal EA (<45 kcal·kg/LBM/day ⁻¹)
	Muia et al. (2016)	56 female athletes (runners) (age: $16-17$ y)	17.9%	Nearly 100% presented as below optimal (<45 kcal·kg/LBM/day ⁻¹) (EA = 36.5 ± 4.5 kcal·kg/LBM/day ⁻¹)
	Reed et al. (2013)	19 Division 1 female soccer players (age: 19.23 ± 0.3 y)	26% at baseline measurement	Average EA was noticeably lower in the middle of the season compared to pre and post season levels
	Schaal et al. (2011)	10 competitive female endurance athletes. Separated into eumenorrheic ($n = 5$, age: 29.8 ± 2.5 y) and amenorrheic ($n = 5$, age: 31.0 ± 4.3 y)	100% in both groups	EA was 29 ± 4.8 kcal.kg/LBM/day in the eumenorrheic group and 18 ± 6.6 kcal.kg/LBM/day in the amenorrheic group
	Schaal et al. (2017)	9 elite female synchronized swimmers (age: 20.4 ± 0.4 y)	100% at baseline measurement	Baseline EA was 25.1 ± 1.8 kcal·kg/LBM/day ⁻¹ and further reduced to 17.8 ± 2.8 kcal·kg/LBM/day ⁻¹ after 4-weeks of intensive training without an increase in EI

Silva & Pavia (2015)	67 rhythmic gymnasts (age: 18.7 ± 2.9 y)	44.80%	EA = 31.5 ± 11.9 kcal·kg/LBM/day ⁻¹ , when separated by age the older group (19-26 y, n = 31) had a lower EA (29.8 ± 10.8 kcal·kg/LBM/day ⁻¹)
Vanheest et al. (2014)	10 junior elite female swimmers (age: 15-17 y). Separated into cyclic (n = 5) and ovarian suppressed (n = 5).	100% of ovarian suppressed subjects.	Over a 12-week training program cyclic were in positive balance in weeks 2 and 4 but had a great EA than ovarian suppressed across the entire study
Viner et al. (2015)	4 female competitive cyclists (age: 38.4 ± 10.3 y)	100% (across a cycling season)	EA was 26.2 ± 14.1 kcal·kg/LBM/day ⁻¹ in pre-season, 25.5 ± 3.1 kcal·kg/LBM/day ⁻¹ in competition season and 23.8 ± 8.9 kcal·kg/LBM/day ⁻¹ during off-season
Woodruff & Meloche (2013)	10 female volleyball players (mean age: 20.9y)	20%	60% had LEA levels below optimal (<45 kcal·kg/LBM/day ⁻¹)

*LEA classified as >30 kcal·kg/LBM/day⁻¹ LBM - Lean body mass, LEA - Low energy availability, EA - Energy availability, EI - Energy intake

Decreased bone mineral density (BMD) is another area of concern for athletes with LEA. Bone formation is suppressed when energy availability (EA) falls under a threshold of 30 kcal.kg/LBM/day⁻¹ (Ihle & Loucks, 2004). When combined with an up-regulation of bone reabsorption, athletes can face serious health consequences including increased short-term risk factors for stress fractures (Barrack, Ackerman, & Gibbs, 2013; Boutroy, Buxsein, Munoz, & Delmas, 2005) or more long-term damage resulting in osteoporosis (Golden & Abrams, 2014). In a study of 29 healthy females, Ihle and Loucks (2004) found that even in short-term phases of energy restriction (as little as 5-days) bone reabsorption was increased and bone formation suppressed when EA fell below a threshold of 30 kcal.kg/LBM/day⁻¹. Left untreated this could lead to long-term reductions in BMD.

Effects of LEA on athletic performance

Logue et al. (2018) determined that depletion of glycogen stores (associated with a decrease in energy intake [EI]) impairs physical performance, as well as mental capacity, by increasing the risk of dehydration, increasing circulating lactate and overall reducing aerobic and strength-based performance. Decreased levels of performance in the exercise protocol (walking on a treadmill) were recorded after a depletion of blood glucose levels for as little as 5-days in 29 habitually sedentary females (Loucks & Thuma, 2003). The same has been shown in athletes over a longer period of decreased EA. In a study of swimmers, a 9.8% decline in 400 m swim time was recorded over a 12-week training season in ovarian-suppressed athletes

compared with an 8.2% increase in performance in their normally menstruating counterparts (VanHeest et al., 2014). VanHeest (2014) also confirms the questioned link between ovarian suppression and LEA, results showed a strong correlation between energy availability and total triiodothyronine (a measure often used to assess thyroid function) ($r = 0.76$). Moreover, LEA may exacerbate poor sports performance concomitantly through loss of lean body mass, decreased RMR, and electrolyte imbalances (El Ghoch, Soave, Calugi, & Dalle Grave, 2013).

Screening techniques in the literature

In order to diagnose LEA, both energy intake and energy expenditure need to be quantified, supplemented with additional measurements of bone mineral density, body composition, and menstrual function (Logue et al., 2018). Thresholds in the literature for determining LEA can range from 30 kcal.kg/LBM/day⁻¹ – 45 kcal.kg/LBM/day⁻¹ (Table 2.). Methodology surrounding the measurement of energy expenditure and intake is variable in the literature (De Souza et al., 1998; Gibbs, Williams, & De Souza, 2013; J. Reed et al., 2013; Silva & Paiva, 2015; VanHeest et al., 2014). Inaccurate measurement of daily expenditure could result in the under or over-estimation of required energy intake to maintain a healthy energy balance, putting subjects at risk of LEA if sustained for an extended period. Using RMR as a measure for energy availability could be more sustainable than measurement of energy intake and expenditure outside a laboratory due to the unreliable nature of self-reported food and activity logs (Donahoo, Levine, & Melanson, 2004).

Researchers (Harris & Benedict, 1918; Mifflin et al., 1990) have developed predictive equations by which an individual can predict their own RMR by inserting individual data, which may prove to be valuable in the field for practitioners without metabolic gas exchange equipment or access to biomarkers. Studies have shown that predictive equations may be useful in large scale studies that require a cost-effective method of measuring RMR (Flack, Siders, Johnson, & Roemmich, 2016; Hasson, Howe, Jones, & Freedson, 2011), although validity in assessing RMR through predictive equations in an athletic population is yet to be confirmed.

Section Three: The Crossroads of LEA and ID in Female Athletes

When combined with LEA, data indicates the effects of low iron stores could be exacerbated. Athletes with low energy intake ($< 2000 \text{ kcal} \cdot \text{day}^{-1}$) have been associated with a higher risk of iron deficiency (Economos, Bortz, & Nelson, 1993). DellaValle and colleagues (2011, 2012) investigated performance times of female collegiate rowers, who were energy deficient (ED) and iron deficient (without anemia), and those with ED and ID were reported to have slower 4 km time-trial times, lower $\text{VO}_{2\text{peak}}$, and reduced energy efficiency compared than athletes that were energy replete. Research is now emerging to determine the dual effects that the crossroads of ID and LEA pose on women, and specifically female athletes. In a review by Petkus et al. (2017) this crossroad was thoroughly examined concluding that iron deficiency may further potentiate the hypometabolic state that is induced by LEA. Iron deficiency may further compromise reproductive health, impair bone development, and impair athletic

performance due to interacting effects that induce hypothyroidism (Goncalves, Resende, Fernandes, & da Costa, 2006; Haouari et al., 1994; Harvey et al., 2005; Moschonis et al., 2013; Toxqui & Vaquero, 2015).

Section Four: Conclusions/Recommendations for Further Research

This review highlights the potential issues that female athletes (especially but not limited to, female endurance athletes) are facing as they participate in larger volumes of intensive training. Most research to date focuses on sedentary or recreationally-active females with a small cohort of research being presented on high-performance athletes. Here lies a gap where females that are highly-trained but not classified as professional fall. The current literature demonstrates that health and performance concerns due to a reduction in energy availability and/or reduced iron stores are present in female athletes, however there is very little agreement on how these issues can be identified and diagnosed. While there is basic screening in elite high-performance cohorts, where health professionals are trained to understand the demands of performing at a high level in the sporting arena, non-professional athletes and exercisers have no such support. Further research should aim to establish thresholds to identify health issues such as energy and iron deficiency in athletic populations. Furthermore, these thresholds should be established in both the clinical and research settings to ensure female athletes are able to train and exercise to their potential, in a healthy and sustainable way. More research is needed to assist health professionals in the identification of exercise and nutrition induced health problems

in female athletes, and to expand the knowledge and education of the athletes themselves.

Chapter 2:

Study 1: The validity of resting metabolic rate (RMR)-prediction equations and reliability of the Parvo Medics TrueOne analyser for measuring RMR in female athletes

This chapter appears in the same format that was required by the journal *Performance Enhancement & Health* where it has been submitted for publication.

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Abstract

Purpose: The aim of the current study was to; 1) assess the test-retest reliability of an indirect calorimetry analyser (Parvo Medics TrueOne), and 2) compare measured RMR with three RMR-predictive (pRMR) equations in female athletes. **Methods:** In part one, 12 recreationally-exercising women (mean \pm SD; age 27.5 ± 12.3 y) performed two RMR assessments, on separate days, utilising the Parvo Medics TrueOne analyser. In part two, 25 women, ranging from recreationally-exercising to highly-trained athletes (mean \pm SD; age 30.1 ± 10.2 y) underwent an RMR assessment using the Parvo Medics TrueOne analyser, which was compared to three calculated pRMR equations (Harris-Benedict (H-B), Mifflin-St Jeor (M), World Health Organisation (WHO)). **Results:** Test-retest reliability for the TrueOne analyser was deemed acceptable (CV = 5.3%, ICC = 0.92). The validity of pRMR when compared to measured RMR showed low levels of agreement in all 3 predictive equations (M: CV = 21.4%, TEE = $269 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.16$, WHO: CV = 21.5%, TEE = $270 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.13$ H-B: CV = 21.6%, TEE = $270 \text{ kcal}\cdot\text{day}^{-1}$, $r = 0.13$). **Conclusion:** The Parvo Medics TrueOne analyser is a reliable tool for measuring RMR. Caution should be taken when using pRMR equations in female athletes, however, in sub-elite female athlete populations, predictive equations may be more accurate than in their lesser-trained counterparts.

KEYWORDS: *resting metabolic rate; energy deficiency; REDs; Harris-Benedict; Mifflin St Jeor*

1. Introduction

Disturbances in energy balance resulting in a state of energy deficiency can compromise overall health status (Mountjoy et al., 2014) and may have a negative impact on sport performance in athletic populations (Loucks et al., 2011). Accurate identification of energy deficiency typically involves the measurement of resting metabolic rate (RMR) often in conjunction with hormonal profiling (De Souza et al., 2007) and energy balance monitoring (Brown, Howatson, Quin, Redding, & Stevenson, 2016). Given that RMR is a commonly used assessment tool (Crenshaw, 2009; Melin et al., 2015; J. Reed, De Souza, Mallinson, Scheid, & Williams, 2015) for the identification of energy deficiency in athletes, the accuracy and reliability of the assessment is critical.

RMR measured by indirect calorimetry provides information at rest by measuring the volume of oxygen consumption (VO_2), volume of carbon dioxide production (VCO_2), and respiratory exchange ratio ($RER = VCO_2 / VO_2$). RMR values can then be used in combination with hormonal profile and energy balance measures to formulate an overall picture of energy availability. Energy availability describes the balance between energy in and energy expenditure, thus the validity and reliability of RMR measurement tools is crucial. The Parvo Medics TrueOne analyser (TrueOne 2400, Parvo Medics, Sandy UT) is often used in sport science laboratory settings to measure RMR as it has been reported to be a valid measurement tool for assessing RMR against the Deltatrac II (VIASYS Healthcare, Inc., SensorMedics, Yorba Linda, CA)

(Cooper et al., 2009) and the Douglas Bag system (Woods, Garvican-Lewis, Rice, & Thompson, 2016) in male recreational and elite athletic populations. However, the test-retest reliability of the TrueOne in the recreationally trained female athletic populations is yet to be determined.

The use of pRMR equations is extensive in metabolic research literature as these tests are inexpensive and easy to administer. The validity of the pRMR equations comes into question when deviating from the original population from which they were developed (Bonganha et al., 2013). For example, the three most frequently used pRMR equations are the Harris-Benedict, the Mifflin-St Jeor, and the World Health Organisation (WHO) equations. The Harris-Benedict equation was developed and validated in the early 1900's. For the validation, 136 male (age: 27 ± 9 y) and 103 female participants (age: 31 ± 14 y) took part in the (initial) study (Harris & Benedict, 1918) and the formula has been studied extensively ever since. The Mifflin-St Jeor equation was derived from a sample of 498 (female $n=247$, male $n=251$) average-weight ($f = 54.9 \pm 4.5$ kg, $m = 68.5 \pm 5.8$ kg), overweight ($f = 63.7 \pm 5.5$ kg, $m = 80.2 \pm 7.5$ kg), obese ($f = 76.2 \pm 6.6$ kg, $m = 92.2 \pm 7.3$ kg), and severely obese ($f = 89.4 \pm 11.0$ kg, $m = 108.7 \pm 13.1$ kg) with individuals aged from 19 to 78 years (45 ± 14 y). The World Health Organisation (WHO) equation was developed using data mostly derived from young European military and police recruits, including 2,279 men and 247 women with 45% of the cohort of Italian descent. While all three RMR-prediction equations have been studied extensively for both their validity and reliability, across

a range of populations, the equations have yet to be validated against measured RMR values in a recreationally trained female-athlete population. The aim of the current study was to determine the reliability of the Parvo Medics TrueOne indirect calorimetry analyser for measuring RMR in a recreationally active female population. A secondary aim of the study was to assess the validity of three pRMR equations relative to the measured RMR values in both recreationally active female athletes and highly-trained, non-professional female athletes.

2. Methods

2.1 Participants

Part one (measured RMR): Twelve recreationally trained females ($\text{VO}_{2\text{max}}$; 40.2 ± 5.6 mL·kg/min⁻¹) (mean \pm SD; age 27.5 ± 12.3 y, weekly exercise duration 8.0 ± 3.6 h·wk⁻¹, height 169.3 ± 7.3 cm, body mass 69 ± 9.4 kg, body mass index 24 ± 3) volunteered to participate in the study.

Part two (measured RMR and predicted RMR): Twenty-five female athletes (recreationally active and highly-trained) volunteered to participate in this study. Criteria for inclusion as a recreationally trained female comprised of being free of any illness or injury and a minimum exercise requirement of at least 3 h·wk⁻¹. Inclusion criteria for the sub-elite population comprised of being free of any illness or injury, be currently regularly training (>10 h·wk⁻¹), and having represented New Zealand in their given sport in the last 3 y. The two groups comprised of recreational female

athletes (n=12) (mean \pm SD; age 27.5 ± 12.3 y, weekly exercise duration 8.0 ± 3.6 h.wk⁻¹, height 169.3 ± 7.3 cm, body mass 69 ± 9.4 kg, body mass index 24 ± 3) and sub-elite female athletes (n=13) (mean \pm SD; age 32.5 ± 7.4 y, weekly exercise duration 16.0 ± 4.0 hr.wk⁻¹, height 167.9 ± 5.8 cm, body mass 60.9 ± 6.7 kg, body mass index 22 ± 2) (see Table 3.) All participants provided informed written consent and the study received ethical approval from the University of Waikato Human Research Ethics Committee (HREC (Health) 2017 #13).

2.2 Study design

Part I:

On two separate occasions, 12 recreationally trained female athletes participated in a test-retest reliability study, performing RMR measurements in our laboratory, with each trial separated by a minimum of 24 h and a maximum of 5 days.

Part II:

Twenty-five female athletes (recreational (n = 12) to sub-elite (n = 13)) performed one RMR assessment using the Parvo Medics TrueOne analyser. The measured RMR was then compared to three pRMR equations (Harris-Benedict, Mifflin St-Jeor and World Health Organisation).

Table 3. Participant characteristics grouped by training status.

	Recreational Athletes (n=12)	Sub-Elite Athletes (n=13)
Age (y)	27.5 ± 12.3	32.5 ± 7.4
Height (cm)	169.3 ± 7.3	167.9 ± 7.8
Body Mass (kg)	69 ± 9.4	60.9 ± 6.7
Body Mass Index	24 ± 3	22 ± 2
Weekly Exercise Duration (h·wk ⁻¹)	8.0 ± 3.6	16.0 ± 4.0

Data shown as mean ± SD.

2.3 Measurement

Measured RMR was conducted using the 'RMR' function on the TrueOne metabolic analyser (TrueOne 2400, Parvo Medics, Sandy UT). On the morning of the assessments, participants arrived at the laboratory for their scheduled lab appointment (between 0600 and 0830 h) following an overnight fast of >10 h, with participants refraining from caffeine and alcohol intake >24 h prior to testing. Participants were instructed to sleep (at least) 8 h the night before testing and recorded their 24 h dietary intake prior to the first trial to be replicated prior to the second trial. Participants were also asked to refrain from exercise in the 24 h leading up to each RMR assessment. Both trials were performed at the same time of day to control for any diurnal variation. Participants were to arrive at the laboratory in a relaxed and rested state and were instructed to perform minimal activity between waking and arrival to the RMR testing session. Upon arrival to the laboratory participants height (cm) and body mass (kg; in minimal clothing) were measured before a 5-min passive rest period prior to the RMR assessment. (Compher, Frankenfield, Keim, Roth-Yousey, & Group, 2006).

2.4 Protocols

Participants were asked to relax and lie supine on a bed with the head rest set at an incline of 45° during the 20-min assessment period, but to maintain alertness with eyes open. The canopy (Figure 1) was then placed over the head, shoulders and upper chest of the participant to reduce contaminant air entering or expired air escaping

during measurement. Flow rate was established at 28 to 30 ml·min⁻¹ within the first 4 min of the assessment as per manufacturer's instructions. Expired air was sampled every 15 s. Data was averaged over 30 s and the participants file was exported in Microsoft Excel format for subsequent calculation of RMR (kcal·day⁻¹). RMR was calculated using the average of the final minute of each 5-min segment of the assessment (4 to 5, 9 to 10, 14 to 15 and 19 to 20 min).



Figure 1. The resting metabolic rate (RMR) assessment setup with the Parvo.

2.5 RMR-predictive equations (pRMR)

Three RMR-prediction (pRMR) equations were calculated in the current study for comparison to the measured RMR value. The timeframe with the highest level of reliability during the RMR assessment (e.g. 4-5, 9-10, 14-15, 19-20 min) was used for comparison to the pRMR equations. All pRMR equations listed have been described elsewhere (Harris & Benedict, 1918; James, 1985; Mifflin et al., 1990; Ramirez-Marrero, Edens, Joyner, & Curry, 2014).

The Harris Benedict equation was calculated as follows:

$$\text{Eq. 1) RMR} = 655 + (9.6 \times \text{BM}) + (1.7 \times \text{H}) - (4.4 \times \text{A})$$

The Mifflin-St Jeor equation was calculated as follows:

$$\text{Eq. 2) RMR} = (9.99 \times \text{BM}) + (6.25 \times \text{H}) - (4.92 \times \text{A}) - 161$$

The World Health Organisation equation was calculated as follows:

$$\text{Eq. 3) RMR} = (13.3 \times \text{BM} + 334 \times (\text{H}/100)) + 35$$

Where BM = body mass (kg), H = height (cm) and A = age (y)

2.6 Statistical analysis

All data is presented as mean \pm SD unless stated otherwise. Statistical significance was set at $p < 0.05$. Comparison of the pRMR with measured RMR assessment was achieved using a Students' paired t-test, Pearson product-moment correlation analysis, 95% limits of agreement (LOA), mean bias (%), and typical error of estimate (TEE). While correlation analysis indicates the degree to which two variables are associated, it does not necessarily indicate the extent to which values agree or disagree. To overcome this limitation, the approach of quantifying the level of agreement between the methods measuring the same parameter (in this case RMR) was employed (Altman & Bland, 1983; Atkinson & Nevill, 1998). The mean difference between methods (2 standard deviations or 95% of a normally distributed population) was determined. The TEE and mean bias between methods was determined using an

excel spreadsheet (Hopkins, 2017), with the TEE expressed both in raw units and as a coefficient of variation (CV) percentage. The magnitude of correlation between the predicted and actual RMR values was assessed with the following thresholds: <0.1, *trivial*; <0.1 to 0.3, *small*; <0.3 to 0.5, *moderate*; <0.5 to 0.7, *large*; <0.7 to 0.9, *very large*; and <0.9 to 1.0, *almost perfect*.

Test-retest reliability data was analysed using an Excel spreadsheet for reliability (Hopkins, 2017). TEE and overall reliability of RMR is presented as coefficient of variation (CV%) and as an absolute value (kcal·day⁻¹) along with intra-class correlation coefficients (ICC's), and upper and lower 90% confidence intervals (CI).

3. Results

3.1 Reliability of RMR measurement

RMR was observed as an *almost perfect* agreement between trials 1 and 2 for the 19-20 min timeframe of the test (TEE = 62 kcal·day⁻¹, CV = 5.3%, ICC = 0.92, Table 4). RMR measures were less reliable in the 0-15 min timeframes, with CV's ranging from 7.0-11.1% (Table 4).

3.2 Validity of RMR-prediction equations

The validity of all three predictive equations when compared to measured RMR was questionable, with low levels of agreement (Table 5). Mifflin-St Jeor showed only a

small level of agreement (CV = 21.4%, TEE = 269 kcal·day⁻¹, $r = 0.16$), followed closely by the WHO equation (CV = 21.5%, TEE = 270 kcal·day⁻¹, $r = 0.13$) and the Harris-Benedict (CV = 21.6%, TEE = 270 kcal·day⁻¹, $r = 0.13$). Bland-Altman plots for agreement between the three predictive equations and measured RMR are shown in Figure 2.

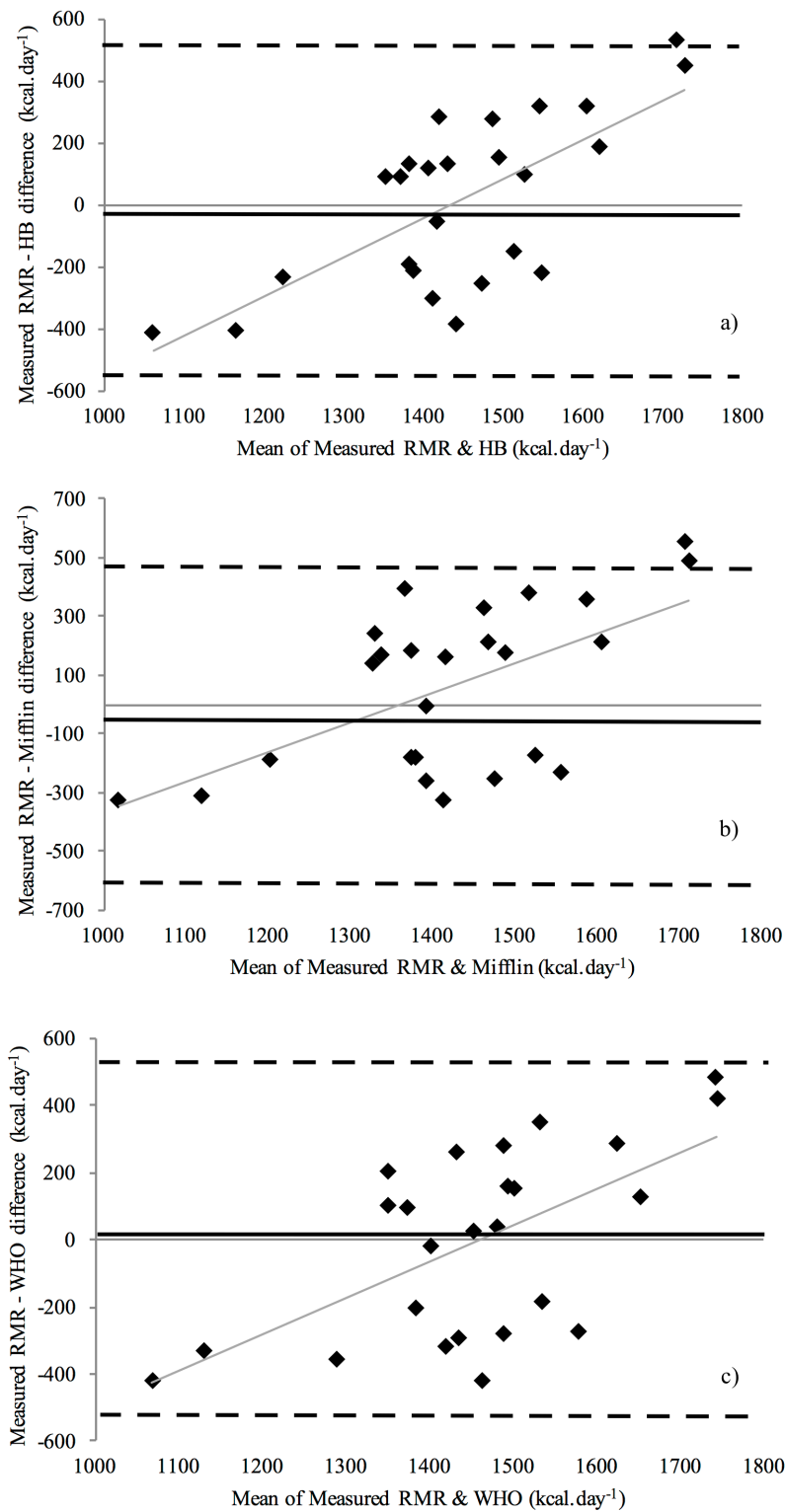


Figure 2. The level of agreement plots (Bland-Altman) showing $\pm 95\%$ limits of agreement (represented as dashed lines) between the measured RMR and each of the RMR prediction equation values in all female athletes (recreational to sub-elite): (a) RMR vs. Harris-Benedict (HB); b) RMR vs. Mifflin-St Jeor (Mifflin); c) RMR vs. World Health Organisation (WHO)). Solid black line represents the difference between methods. Grey line represents linear line of best fit.

Data was then separated to examine training status effect of two groups: recreationally trained female athletes (n=12) and; sub-elite female athletes (n=13). Results are presented in Table 6. Between groups there was a significant difference in measured RMR values ($p < 0.01$). In recreationally trained female athletes, the equation that showed the highest agreement to measured RMR was the Mifflin-St Jeor equation (CV = 12.9%, TEE = 148 kcal·day⁻¹, $r = 0.69$), followed by the WHO equation (CV = 13.0%, TEE = 150 kcal·day⁻¹, $r = 0.68$) and then the Harris-Benedict (CV = 14.5%, TEE = 162 kcal·day⁻¹, $r = 0.61$) (Table 6). In sub-elite female athletes, the equation that showed the highest agreement to measured RMR was the Mifflin-St Jeor (CV = 8.1%, TEE = 131 kcal·day⁻¹, $r = 0.75$), followed the WHO (CV = 8.5%, TEE = 138 kcal·day⁻¹, $r = 0.72$) and the Harris-Benedict (CV = 8.5%, TEE = 139 kcal·day⁻¹, $r = 0.71$).

Table 4. Test-retest reliability outcomes for resting metabolic rate (RMR) expressed as typical error of estimate (TEE), coefficient of variation (CV%) and Intra-class correlation coefficient (ICC).

	RMR (kcal·day ⁻¹)		TEE (kcal·day ⁻¹) (90% CI)	CV% (90% CI)	ICC (90% CI)
	Trial 1 (Mean ± SD)	Trial 2 (Mean ± SD)			
4 to 5-min	1332 ± 243	1253 ± 227	143 (107 - 222)	10.5 (7.8 – 16.8)	0.66 (0.27 - 0.86)
9 to 10-min	1227 ± 175	1307 ± 192	88 (66 - 137)	7.0 (5.2 – 11.1)	0.80 (0.53 - 0.93)
14 to 15-min	1216 ± 229	1273 ± 222	126 (94 - 196)	11.1 (8.2 – 17.7)	0.73 (0.38 - 0.89)
19 to 20-min	1244 ± 207	1285 ± 194	62 (42 – 96)	5.3 (3.9- 8.3)	0.92 (0.80 - 0.97)

Table 5. Comparison of measured RMR and three RMR-prediction equations in all female athletes (recreational to sub-elite). Mean bias, range of mean difference (95% limits of agreement or $\pm 2SD$) typical error of estimate (TEE – raw and CV%) and Pearson's correlation coefficient (r) between methods.

	RMR (kcal·day ⁻¹) (Mean \pm SD)	Mean bias (kcal·day ⁻¹) (90% CI)	Range of mean difference (kcal·day ⁻¹) ($\pm 2SD$)	TEE (kcal·day ⁻¹) (90% CI)	CV% (90% CI)	Correlation (r) (90% CI)
Measured RMR	1452 \pm 267	-	-	-	-	-
Harris-Benedict	1438 \pm 113	-14 (-108 - 80)	-554 - 526	270 (218 - 358)	21.6 (17.1 – 29.5)	0.13 (-0.22 - 0.45)
Mifflin-St Jeor	1392 \pm 140	-61 (-157 - 36)	-611 - 490	269 (217 - 356)	21.4 (17.0 – 29.4)	0.16 (-0.19 – 0.47)
World Health Organisation	1460 \pm 133	7 (-89 - 104)	-544 - 559	270 (218 - 358)	21.5 (17.1 – 29.5)	0.13 (-0.21- 0.45)

Table 6. Comparison of measured RMR and three RMR-prediction equations in sub-elite athletes and recreational athletes. Typical error of estimate (TEE – raw and CV%) and Pearson’s correlation coefficient (*r*) between methods.

	RMR (kcal·day ⁻¹) (Mean ± SD)		Mean bias (kcal·day ⁻¹) (90% CI)		TEE (kcal·day ⁻¹) (90% CI)		CV% (90% CI)		Correlation (<i>r</i>) (90% CI)	
	<i>Sub-Elite</i>	<i>Recreational</i>	<i>Sub-Elite</i>	<i>Recreational</i>	<i>Sub-Elite</i>	<i>Recreational</i>	<i>Sub-Elite</i>	<i>Recreational</i>	<i>Sub-Elite</i>	<i>Recreational</i>
Measured RMR	1629 ± 189*	1260 ± 195*	-	-	-	-	-	-	-	-
Harris-Benedict	1391 ± 81 [#]	1484 ± 128 [#]	-234 (-305 - -163)	224 (144 - 304)	139 (104 - 216)	162 (120 - 259)	8.5 (6.3 – 13.5)	14.5 (10.5 – 24.0)	0.71 (0.35 – 0.89)	0.61 (0.16 – 0.85)
Mifflin-St Jeor	1366 ± 102 [#]	1451 ± 155 [#]	-293 (-358 - -228)	191 (117 - 265)	131 (98 - 203)	148 (109 - 236)	8.1 (6.0 – 12.9)	12.9 (9.4 – 21.3)	0.75 (0.42 – 0.90)	0.69 (0.30 – 0.89)
World Health Organisation	1405 ± 102 [#]	1518 ± 141 [#]	-224 (-291 - -157)	258 (184 - 332)	138 (103 - 213)	150 (111 - 239)	8.5 (6.3 – 13.5)	13.0 (9.5 – 21.5)	0.72 (0.37 – 0.89)	0.68 (0.28 – 0.88)

* represents significant difference between groups (*p* < 0.01). [#] represents significant difference when compared to Measured RMR (*p* < 0.01).

4. Discussion

The current study assessed the reliability of the Parvo Medics TrueOne analyser as a method of indirect calorimetry for assessing RMR in a recreationally trained female athlete population. The study also compared measured RMR values to those obtained through three commonly used pRMR equations in 25 female athletes. The main findings of this study were a) the Parvo Medics TrueOne analyser resulted in *almost perfect* levels of test-retest reliability and; b) the three pRMR equations analysed in the present study overestimated the RMR in the recreationally trained female athlete cohort, however, in the sub-elite population of female athletes, the predictive equations underestimated RMR and showed a higher correlation.

The test-retest reliability of the Parvo Medics TrueOne analyser for measuring RMR in the current study is in agreement with previous studies; Woods and colleagues (2016) reported a test-retest reliability CV of ~6.5 % for the same analyser in a mixed population of male and female endurance athletes. Woods et al. (2016) employed an onsite protocol to minimize the influence of activities of daily living, such as rising from bed, dressing and transport to the laboratory (e.g. participants performed RMR measures on waking). While this was not performed, and is a potential limitation to the current study, it is interesting to note that our study resulted in higher levels of reliability. This may be related to the differences in protocol used (e.g. mouthpiece in exercise mode vs. canopy/hood in RMR mode); or potentially, the studied population all being exercisers and a more uniform cohort (mixed vs. single sex only). Similar to the current study, Cooper et

al. (2009) reported a CV of 4.8% for test-retest reliability of the Parvo Medics TrueOne analyser.

Compher et al. (2006) recommends that a CV <10% is sufficient for the measurement of resting energy status. Based on this recommendation, the pRMR equations used in the current study were inappropriate for estimating RMR in the female recreational athletes, however, in sub-elite female athletes, the equations were valid in estimating RMR. Results from the recreational female athletes in our study produced CV's of between 12.9 – 14.0% when compared to our indirect calorimetry analyser, with all three equations over-predicting RMR values. The Mifflin-St Jeor equation was the closest predictive value, with a CV of 12.9% and a mean bias of 191 kcal·day⁻¹.

The pRMR equations underestimated RMR by 131-139 kcal·day⁻¹ in the sub-elite female athletes. The current findings align with observations of Flack and colleagues (2016) which indicated that as fat free mass increased, the predictive equations further underestimated RMR which may explain the underestimation of the sub-elite female athlete population. Although fat-free mass was not measured in the current study, it is assumed, due to the literature on body composition and female athletes, that a greater percentage of body mass is of lean muscle in this cohort. Hasson et al. (2011) also observed an underestimation using the Mifflin-St Jeor equation when participants were classified as within a normal weight range in both males and females.

In other research by Bullough, Gillette, Harris, and Melby (1995) it has been reported that people who expend large amounts of energy and also match this expenditure with large amounts of energy intake were shown to have an elevated RMR. Conversely, RMR is reduced when large amounts of energy is expended and energy intake is decreased. Tremblay et al. (1986) also demonstrated this relationship of low energy intake suppressing overall RMR. This could explain the significantly lower measured RMR in our recreationally-exercising females compared to highly-trained female athletes however we did not measure energy intake or expenditure in either population. With this knowledge, it should be noted that pRMR equations do not take into account energy intake or expenditure, which may contribute to the high variance between different populations using the equations.

A common element of all three prediction equations utilized for comparison in this study is they do not take into account physical activity (Weijs, 2008). The Harris-Benedict equation was developed and validated using predominantly white participants (136 male and 103 female) of an average build and body mass (Harris & Benedict, 1918). The Mifflin-St Jeor equation was derived from a sample of 498 average-weight, overweight, obese, and severely obese individuals aged 19 to 78 (Mifflin et al., 1990), and the WHO equation was mostly derived from young European military and police recruits, including 2,279 men and 247 women with 45% of Italian descent (James, 1985). It is known that greater levels of physical activity increase energy expenditure and lean mass, factors which have been shown to effect RMR. Thus, the importance of testing the validity of these well-

established equations in cohorts of varying physical activity levels, as demonstrated by the cohorts of the current study.

The Parvo Medics TrueOne analyser is a reliable tool for measuring RMR in recreationally trained female athletes. However, the over-prediction of RMR derived from pRMR equations in recreationally trained female athletes, when compared to measured RMR values, is a factor to consider when investigating energy balance and energy availability. It is important to note also that all the pRMR equations showed a significant difference to measured RMR, this could suggest a need for new predictive tools that are more accurate to specific populations. As RMR is a tool used in the competitive sporting environment to determine energy availability and subsequent prevention of relative energy deficiency in sport (REDs); additional research in sub-elite female athletes is warranted to validate the predictive equations in this specific population.

Chapter 3:

Study 2: Under the radar: A snapshot health profile of highly-trained, non- professional female athletes

Abstract

Purpose: The aim of the current study was to investigate the haematological profiles of non-professional highly-trained female athletes. **Methods:** 13 non-professional highly-trained female endurance athletes provided a blood test where iron markers of haemoglobin (Hb), haematocrit (Hct), C-reactive protein (Crp), serum iron, serum ferritin and transferrin were assessed. Methodology also included; resting metabolic rate (RMR) assessment, Dual-energy x-ray absorptiometry (DXA) scans, diet and training diary, and menstrual cycle tracking to assess other female health risks associated with relative energy deficiency in sport (REDs). **Results:** Six of the 13 females were classified as iron deficient (ID). Serum iron, serum ferritin, and transferrin were all significantly higher in the non-iron deficient (non-ID) group when compared to the iron deficient (ID) group ($p < 0.05$). Haemoglobin (Hb) and Haematocrit (Hct) were significantly lower in the ID group compared with the non-ID group ($p < 0.05$). Crp and serum iron showed a large negative correlation ($r = -0.66$), Crp and serum ferritin a very large negative correlation ($r = -0.72$) and transferrin presented a large correlation with Crp ($r = 0.70$). **Conclusion:** 46% of this population were diagnosed with iron levels below optimal; this could have lasting health effects and impair athletic performance. The need for more education and support in non-professional athletes regarding iron deficiency is strongly advised.

Introduction

Athletes are at an increased risk of iron deficiency due to exaggerated losses through haemolysis, sweating, gastrointestinal bleeding, and exercise-induced acute inflammation (Auersperger et al., 2013; Ma et al., 2013; Peeling et al., 2008). Female athletes are at further risk due to menstrual blood losses and lower dietary iron intake (Beard & Tobin, 2000; Hinton, 2014). While many high-performance athletes have access to medical professionals to diagnose and treat iron deficiency (with and without anaemia), the majority of non-professional female athletes do not, increasing the risk of chronic iron deficiency and the negative health and performance implications (Beard, 2001; Nazem & Ackerman, 2012; Petkus et al., 2017; Wade & Jones, 2004). Exactly how prevalent iron deficiency is among female athletes is not known, but some research suggests this number could be as high as 50% in endurance athletes (Hinton, 2014). According to the USA Department of Health & Human Services (2017), the prevalence of iron deficiency in the general premenopausal female population is roughly 9%. A 2011 study of female collegiate rowers found 10% of the athletes were anaemic and 30% had iron stores below optimal level (DellaValle & Haas, 2011).

The increased incidence of iron deficiency in female endurance athletes is thought to be the result of low dietary iron intake in this population, losses of iron in menstrual blood, sweat iron loss, and gastrointestinal blood loss (McClung et al., 2014). However, it may also be related to acute exercise-induced inflammation (Peeling, 2010). Hepcidin, an iron-regulatory hormone, has been identified as an enzyme of interest in the interplay of inflammation and iron absorption

(Auersperger et al., 2012; McClung et al., 2013; Newlin et al., 2012). A single session of exercise not only increases inflammatory cytokines, but also elevates oxidative stress (Newlin et al., 2012). Post-exercise inflammation has been shown to up-regulate hepcidin, which acts to inhibit the absorption of iron (Di Santolo et al., 2008; Newlin et al., 2012; Peeling et al., 2009b; Roecker, Meier-Buttermilch, Brechtel, Nemeth, & Ganz, 2005; Sim et al., 2013; Sim et al., 2012). Hepcidin acts to block iron efflux from various cell types by binding to the iron transporter ferroportin and down-regulating its expression, in both inflammatory states and in response to increased levels of circulating iron (Nicolas et al., 2002). Among inflammatory markers, Crp is the most clinically useful and the best markers of inflammation, and is often used in athletic populations as chronic low grade inflammation is associated with prolonged exercise (Kazeem, Olubayo, & Ganiyu, 2012).

Female athletes participating in regular training programmes with insufficient caloric intake are at risk of a state of chronic low energy availability (LEA). This LEA can also contribute to stage 1 iron deficiency (Suominen, Punnonen, Rajamaki, & Irjala, 1998) and may contribute to menstrual cycle dysfunction, compromised reproductive function, impaired bone health and thyroid function, and impaired athletic performance (Petkus et al., 2017).

The aim of the current study was to investigate prevalence of iron deficiency in a non-professional highly-trained population of female endurance athletes. As the research in this cohort is limited, we aimed to get a snapshot of their current energy availability and hormone health.

Methods

Participants

Twenty non-professional, highly-trained, premenopausal female endurance athletes (mean \pm SD: age: 32 ± 7 y, training volume: 18.5 ± 4.1 hrs) volunteered to participate in this study. Women were initially excluded if they did not meet the following criteria: a) regular menstrual cycles (24-35 days in length) or using a contraceptive (monophasic pill or intrauterine device) for >6 months before entering the study, b) represented New Zealand at an elite level within the past three years and currently participating on a regular basis in competitive endurance-sport activity (running, cycling, triathlon), c) have no current or recent (<3 months) injury or illness, or chronic disease, d) are not pregnant or not planning to become pregnant, and e) non-smoking. All participants provided informed written consent and the study received ethical approval from the Institutions Human Research Ethics Committee.

Food & training diaries

Participants recorded all diet and fluid intake, and training over seven days on an online diet program (MyFitnessPal, Under Armour, Baltimore, MD, USA) and exercise (TrainingPeaks, Boulder, CO, USA) platforms. They were provided with verbal and written instructions for accurate recording of all foods and fluids consumed. Once the subjects submitted their diaries, a dietitian checked the food records and confirmed the contents, clarifying specific items and/or detailed information as necessary. Dietary analysis of the food diaries was conducted using

food analysis software (NutriBase Pro 17, CyberSoft Inc, Phoenix, AZ, USA). Training hours at intensity was classified as all training done above 80% $\text{VO}_{2\text{max}}$ and calculated upon analysis of training logs using heart rate-based training impulse (TRIMP).

Determination of menstrual cycle

Participants used the mobile application HelloClue (Version 3.7.1, BioWink GmbH, Berlin) to keep track of cycle length over the course of three full menstrual cycles. For those individuals using an oral contraceptive pill or intrauterine device, the brand and history of use was noted.

Blood samples

Blood samples were collected following an overnight fast (6:30 p.m. to 8:00 a.m.). All blood samples were obtained via an antecubital vein while in a seated position. After drawing blood, serum and plasma samples were obtained after centrifugation (3000 rpm, 10 min, 4 °C) and stored at –80 °C until analysed.

The sex hormone profile (oestrogen, progesterone, testosterone, follicular stimulating hormone [FSH], luteinizing hormone [LH], and beta-human chorionic gonadotropin [β -hCG]); DHEA-S, cortisol, iron-status variables (iron, ferritin, transferrin), and a marker of inflammation (Crp) were analysed at the local health-board utilised medical laboratory.

Plasma oestrogen was measured via a Sorin (sensitive) RIA kit (Sorin Biomedica Diagnostics, Saluggia, Italy). Progesterone was measured using the commercial

conjugate-antibody method (ELISA kit, Assay Designs, Ann Arbor, MI, USA) with sample concentrations determined against a standard curve. Intra- and inter-assay coefficients of variation, respectively, for the midrange standards were for plasma oestrogen ($115 \text{ pg}\cdot\text{ml}^{-1}$) 2.80% and 6.00%, and progesterone ($1.5 \text{ ng}\cdot\text{ml}^{-1}$) 3.61% and 4.80%. Testosterone, cortisol and DHEA-S concentrations were measured by a sensitive and specific liquid chromatography-tandem mass spectrometry assay with minor modifications to improve assay specificity. Iron-status variables were measured immediately after non-fasting venous blood sampling (antecubital venepuncture into two evacuated tubes with EDTA and serum separator) included haemoglobin, haematocrit, ferritin, iron, transferrin, and Crp.

Dual-energy X-ray absorptiometry (DXA)

A NHANES positioning DXA (Hologic Discovery DXA, Hologic Inc Bedford, MA, USA) scan was used to assess body composition and in particular percent body fat, fat mass, lean body mass and bone mineral density. Participants underwent an anthropometrical analysis of height (to the closest 0.1 cm) using a medical stadiometer (Harpenden, Holtain Limited, Crymych, UK) and mass (to the closest 0.1 kg) on medical scales (WM202, Wedderburn, Bilinga, Australia) prior to undergoing a body composition scan on the DXA. On the morning of the scan, the participant confirmed they had fasted overnight; rested and refrained from strenuous exercise for the previous 24 hrs; wore minimal clothing; bladder voided; as well as jewellery and metal removed, prior to scanning (De Souza et al., 2008; Reed, De Souza, Mallinson, Schied, & Williams, 2015).

Statistical analysis

All data is presented as mean \pm SD unless stated otherwise. Statistical significance was set at $p < 0.05$. Comparison of the ID and non-ID groups was achieved using a Students' unpaired t-test and Cohen's effect sizes (d). The effect sizes were determined using an excel spreadsheet (Hopkins, 2007). The magnitude of effect sizes was assessed with the following thresholds: <0.2 , *trivial*; $0.2-0.6$, *small*; $0.6-1.2$, *moderate*; $1.2-2.0$, *large*; $2.0-4.0$, *very large*; >4.0 , *extremely large* (Hopkins, Marshall, Batterham, & Hanin, 2009). Pearson's correlations (r) were used to examine associations between variables. The magnitude of correlation between variables was assessed with the following thresholds: <0.1 , *trivial*; $<0.1-0.3$, *small*; $<0.3-0.5$, *moderate*; $<0.5-0.7$, *large*; $<0.7-0.9$, *very large*; and $<0.9-1.0$, *almost perfect* (Hopkins, 2002).

Results

Of the 13 women who were included in this study, six were classified as iron deficient (ID) and 7 were classified as non-iron deficient (non-ID) based on a threshold of <30 ug/L serum ferritin indicating ID (Suominen et al., 1998). Dietary intake of iron between groups was not significantly different (ID: 13.99 ± 6.26 ; non-ID: 14.20 ± 5.22 mg \cdot day $^{-1}$). Characteristics for each group are detailed in Table 7. Crp was elevated in the ID group ($p = 0.0001$, $d = -3.33 \pm 0.93$, *very large*), as was transferrin ($p = 0.004$ $d = -1.82 \pm 0.91$, *large*). Serum ferritin ($p = 0.002$, $d = 1.97 \pm 0.92$, *large*), serum iron ($p = 0.011$, $d = 1.55 \pm 0.91$, *large*), haemoglobin (Hb) ($p = 0.036$, $d = 1.24 \pm 0.94$, *large*) and haematocrit (Hct) ($p = 0.034$, $d = 1.27 \pm 0.92$,

large) were all significantly lower in the ID group when compared with the non-ID group (Table 9).

When compared with normative data (Table 8); serum ferritin (ID = 27.5 ± 2.8 ug/L, non-ID = 41 ± 7.3 ug/L), serum iron (ID = 20.5 ± 1.0 mmol/L, non-ID = 25.5 ± 3.4 mmol/L), cortisol (ID = 359.3 ± 112.4 nmol/L, non-ID = 320.8 ± 96.4 nmol/L) and Hb (ID = 130.5 ± 7.8 g/L, non-ID = 143.8 ± 8.4 g/L) were all within normal ranges in both ID and non-ID cohorts. Transferrin was higher than normal in the ID group (3.9 ± 0.1 g/L) but the non-ID group (3.1 ± 0.6 g/L) remained within the normal range. Crp was elevated above the normal range in both ID (2.8 ± 0.5 mg/dL) and non-ID athletes (0.8 ± 0.6 mg/dL).

Correlations between measured variables are shown in Table 10. There was a very large correlation between serum iron and Crp ($r = -0.72$, Fig 3a). Large correlations were also seen between Crp and serum ferritin ($r = -0.66$) and Crp with transferrin ($r = 0.70$). Training hrs·wk⁻¹ showed a large correlation with measured RMR ($r = -0.66$) and intensity hrs·wk⁻¹ correlated well with BMD ($r = 0.68$, Fig 3b). Cortisol and transferrin were also largely correlated ($r = 0.53$, Fig 3c).

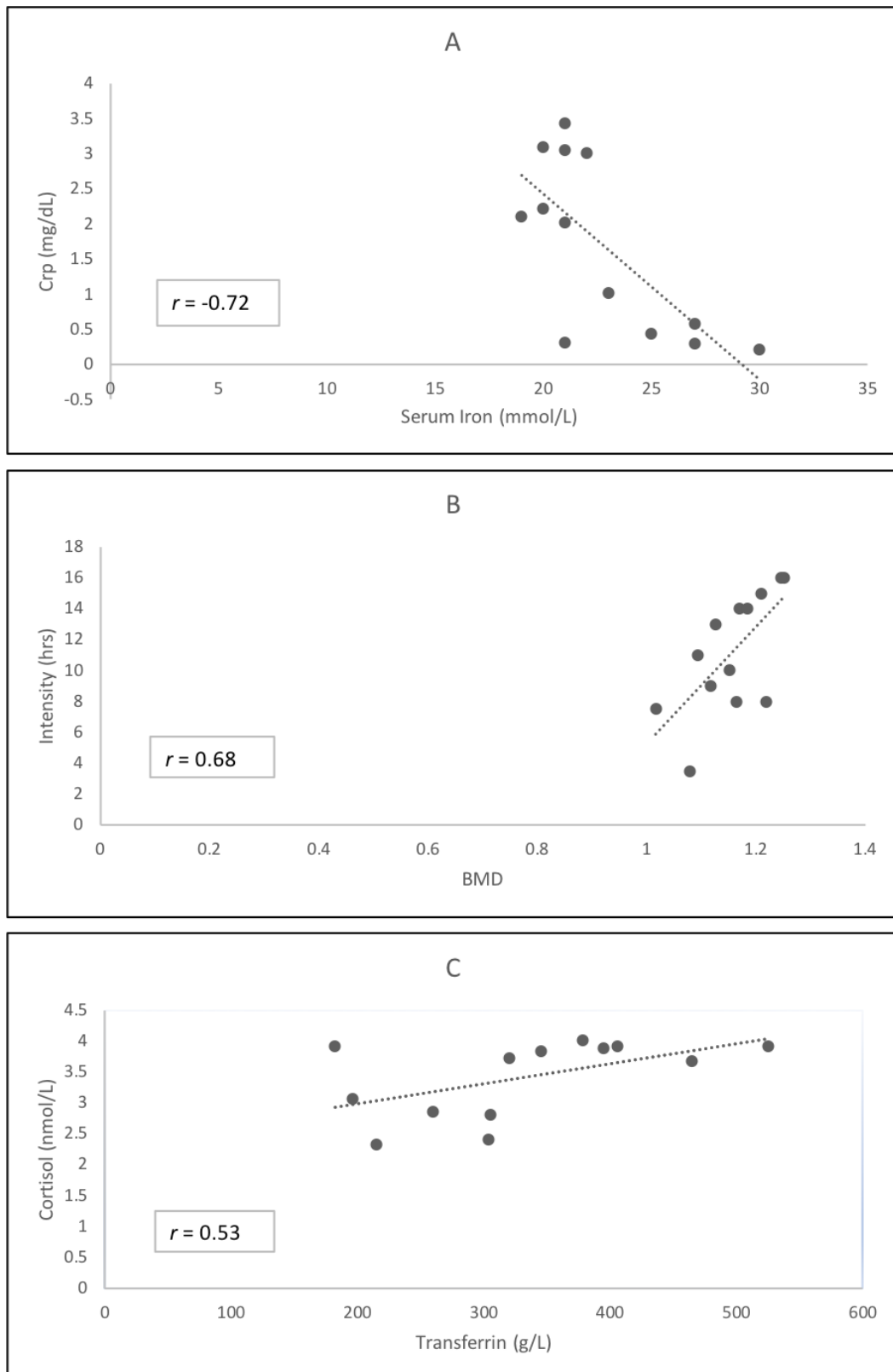


Figure 3. The correlation between health markers in highly-trained female endurance athletes: (a) serum iron vs C-reactive protein (Crp); b) bone mineral density (BMD) vs hours at intensity; c) cortisol vs transferrin).

Table 7. Participant Characteristics (grouped by iron status, iron deficient (ID) & non-iron deficient (non-ID)).

	ID (n=6)	Non ID (n=7)
Age (yrs)	31 ± 3	34 ± 10
Height (cm)	170.7 ± 2.4	164.2 ± 6.8
Body Mass (kg)	62.6 ± 7.5	58.8 ± 5.7
Body Fat (%)	21.0 ± 6.2	18.8 ± 5.0
BMI	21.6 ± 2.3	21.9 ± 1.7
Training (hrs/wk)	17 ± 5	20 ± 3

*No statistically significant differences were found between the two groups (p = <0.05). BMI – body mass index. Data shown as mean ± SD.

Table 8. Iron deficient (ID) and non-iron deficient (non-ID) haematological markers compared to normal ranges.

	ID (mean \pm SD)	Non-ID (mean \pm SD)	Normal Ranges*
Serum Ferritin (ug/L)	27.5 \pm 2.8	41 \pm 7.3	15-200
Serum Iron (mmol/L)	20.5 \pm 1.0	25.5 \pm 3.4	11-29
Transferrin (g/L)	3.9 \pm 0.1	3.1 \pm 0.6	2.1-3.6
Crp (mg/dL)	2.8 \pm 0.5	0.8 \pm 0.6	<0.5
Cortisol (nmol/L)	359.3 \pm 112.4	320.8 \pm 96.4	251-552
Hb (g/L)	130.5 \pm 7.8	143.8 \pm 8.4	120-160

*Normal Ranges as cited in Wians, F. H. (2018). Crp – C-reactive protein, Hb – haemoglobin.

Table 9. Comparison of haematological profiles, bone mineral density (BMD) and resting metabolic rate (RMR) in female iron deficient (ID) athletes and non-iron deficient (non-ID) athletes.

Marker	ID (mean \pm SD)	Non ID (mean \pm SD)	P-Value (p)	Effect Size (mean \pm 90% CI)
Serum Ferritin (ug/L)	27.5 \pm 2.8	41.0 \pm 7.3	0.002*	1.97 \pm 0.92 (large)
Serum Iron (mmol/L)	20.5 \pm 1.0	25.5 \pm 3.4	0.011*	1.55 \pm 0.91 (large)
Transferrin (g/L)	3.9 \pm 0.1	3.1 \pm 0.6	0.004*	-1.82 \pm 0.91 (large)
Crp (mg/dL)	2.8 \pm 0.5	0.8 \pm 0.6	0.0001*	-3.33 \pm 0.93 (very large)
Cortisol (nmol/L)	359.3 \pm 112.4	320.8 \pm 96.4	0.373	-0.47 \pm 0.95 (unclear)
Hb (g/L)	130.5 \pm 7.8	143.8 \pm 8.4	0.036*	1.24 \pm 0.94 (large)
Hct (g/L)	0.392 \pm 0.01	0.430 \pm 0.02	0.034*	1.27 \pm 0.92 (large)
FSH (mU/mL)	4.8 \pm 1.2	22.0 \pm 33.5	0.2952	0.56 \pm 0.90 (unclear)
LH (mU/mL)	6.0 \pm 3.9	17.7 \pm 20.1	0.3099	0.60 \pm 0.92 (unclear)
Preogesterone (ng/mL)	2.5 \pm 1.7	5.0 \pm 4.0	0.306	0.55 \pm 0.99 (unclear)
Oestradiol (pmol/L)	1037.3 \pm 618.6	733.0 \pm 411.4	0.271	-0.67 \pm 1.18 (unclear)
Testosterone (nmol/L)	1.2 \pm 0.8	1.7 \pm 0.6	0.303	0.55 \pm 0.95 (unclear)
BMD	1.2 \pm 0.1	1.2 \pm 0.1	0.7	-0.20 \pm 0.96 (unclear)
RMR (kcal)	1581.7 \pm 84.2	1670.9 \pm 247.9	0.42	0.43 \pm 0.91 (unclear)

*Indicates statistical significance (p < 0.05)

Table 10. Pearson's correlations between measured blood markers, bone mineral density (BMD), and resting metabolic rate (RMR) in highly-trained, non-professional female athletes.

	Body Mass	% BF	RMR	Cortisol	Serum Ferritin	Serum Iron	Transferrin	Crp	BMD	Progesterone	Oestradiol	Age	Hct	Hb	Training hrs/wk
% BF	0.60**														
RMR	0.14	-0.09													
Cortisol	0.41*	-0.01	-0.49*												
Serum Ferritin	-0.21	-0.32*	0.11	0.06											
Serum Iron	0.03	-0.21	0.45*	-0.11	0.70**										
Transferrin	0.40*	0.38*	-0.48*	0.53**	-0.23	-0.45*									
Crp	0.04	0.13	-0.52	0.31*	-0.66**	-0.72***	0.70**								
BMD	0.66**	0.23	0.12	0.48*	0.03	0.16	0.16	-0.11							
Progesterone	0.14	-0.38*	0.14	0.43*	0.58**	0.61**	-0.1	-0.31*	0.46*						
Oestradiol	0.13	0.4	-0.33*	0.09	-0.29	-0.11	0.42*	0.34*	0.33*	-0.53**					
Age	0.02	0.18	-0.20	-0.13	0.37*	0.31*	0	-0.3	-0.37*	0.17	-0.37*				
Hct	-0.24	-0.21	0.08	-0.15	0.81***	0.71***	-0.21	-0.44*	-0.19	0.28	-0.02	0.32*			
Hb	-0.09	-0.13	0.15	-0.22	0.75***	0.74***	-0.22	-0.5	-0.19	0.2	-0.03	0.45*	0.95***		
Training hrs/wk	0.1	-0.23	-	0.31*	0.13	0.04	0.2	0.25	0.06	0.24	0.05	0.26	0.27	0.27	
Intensity hrs/wk	0.3	-0.13	-0.34	0.48*	0.18	0.24	0.15	0.03	0.68**	0.48*	0.21	-0.06	0.1	0.12	0.7

Magnitude of correlation = *Moderate, **Large, ***Very large. BF – body fat, RMR – resting metabolic rate, Crp – C-reactive protein, Hb – Haemoglobin, Hct – Haematocrit, BMD – bone mineral density.

Discussion

While the main outcome of this study was to examine the biochemical profile in a cohort of non-professional highly-trained female athletes, interesting correlations between health markers were also identified that should be further investigated. Based upon the threshold of <30 ug/L serum ferritin indicating early stages of ID (Suominen et al., 1998), 46% (n=6) of the female endurance athletes measured presented with iron deficiency. This is similar to what has been previously reported in other endurance based studies (Constantini et al., 2000; DellaValle & Haas, 2011; McClung, Marchitelli, Friedl, & Young, 2006). While many studies differ in specific identification thresholds for diagnosing iron deficiency, ranging from <12 to <35 ug/L serum ferritin (Burden et al., 2015; Di Santolo et al., 2008; Milic et al., 2011; Pate et al., 1993; Peeling et al., 2007), it has been suggested that athletes, especially endurance trained athletes, are more at risk of iron depletion through increased losses and often inadequate intake. Therefore, the threshold for iron deficiency should be higher to support optimal function and performance (Whiting & Barabash, 2006).

This study provides an insight into the health profiles of a group non-professional highly-trained female endurance athletes. While it has been identified in the literature that female athletes are at risk of health problems related to increased activity levels and/or inadequate diet (Day et al., 2015; De Souza & Williams, 2004; Kim & Nattiv, 2016; McClung et al., 2014), there is a gap in knowledge on highly-trained, non-professional female athletes. Much of the studied population has been recreationally-active females (Alaunyte et al., 2014; Choe et al., 2001; Pate

et al., 1993; Sinclair & Hinton, 2005; Spodaryk et al., 1996) who are not under the same intensive training loads that the athletes of the current study. Alternatively there is also a paucity of research in collegiate/professional athletes (Clement & Sawchuk, 1984; DellaValle & Haas, 2011; Ostojic & Ahmetovic, 2008; J. Reed et al., 2013; Schaal et al., 2017), however they have the benefit of increased awareness of athlete focused health problems and a team of professionals with knowledge of such issues. According to the classification system in Decroix, De Pauw, Foster, and Meeusen (2016), the female athletes in the current study matched the training load of the PL5 category ($>17\text{hrs}\cdot\text{wk}^{-1}$), whereby athletes were described as professional. There is limited research determining the difference between highly-trained athletes and professional athletes, especially in females. However, due to the matched training loads, these athletes may have a higher chance of developing clinical health issues from heavy training loads paired with inadequate support from high-performance support staff (medical, dieticians, physiologists, biomechanists/strength and conditioning coaches, psychologists).

As noted in other iron studies (Ahmadi et al., 2010; Constantini et al., 2000; Di Santolo et al., 2008; Malczewska et al., 2001) we observed an increase in transferrin and a decrease in serum iron, serum ferritin, Hb and Hct in the ID group (stage 1 iron deficiency). Crp was also higher in this group, which supports the current theory that inflammation plays a key role in the absorption of iron (Auersperger et al., 2012; Nicolas et al., 2002; Peeling et al., 2008; Peeling et al., 2009a). Although significantly different between groups ($p < 0.05$), when these

iron parameters were compared with current clinical guidelines (Wians, 2018), none of the participants would have been classified as iron deficient.

Through further analysis of the biochemical profiles in our study we also observed some correlations of interest. Crp showed a strong negative correlation with serum iron and was also well correlated with serum ferritin and transferrin, again supporting claims in recent research that inflammation could have a deleterious effect on iron absorption (Auersperger et al., 2012; Nicolas et al., 2002; Peeling et al., 2008; Peeling et al., 2009a).

One novel concept of the current study was the unique population studied: a non-professional, highly-trained cohort of female endurance athletes. Growing evidence of iron deficiency in athletic populations warrants a re-evaluation of screening and the thresholds that results are being screened against. Our research suggests the female athletes in the current study could be misdiagnosed in normal healthcare settings based on the clinical guidelines that we compared our results to in Table 8. Recent reviews support this finding suggesting there are benefits to the monitoring of iron levels in athletes (Alaunyte, Stojceska, & Plunkett, 2015; Pedlar, Bruignara, Bruinvels, & Burden, 2017) as even the early stages of a deficiency could have a disadvantageous effect on aerobic exercise performance (DellaValle & Haas, 2011).

This research adds to the evident need for an athlete-centred set of guidelines when identifying health issues such as iron deficiency. 46% of the athletes in the current study were identified as iron deficient below levels for optimal function. To avoid long-term health problems and enhance athletic performance, athletes

that are training at high levels below professional status need the same education and diagnosis support as professional elite athletes.

Chapter 4: Conclusion

Summary

Frequently, health-related research is generalized from a less-trained or sedentary population to an elite population, which may only be applicable to the specific population studied. The aim of this thesis was to examine the health status in an under-represented cohort of female athletes and build upon the literature to provide more knowledge and awareness to both practitioners working with female athletes and the athletes themselves. The first study of this thesis reported RMR values for recreational vs elite female athletes; whereby the validity of RMR-predictive equations and laboratory techniques were examined in both populations. The second study of this thesis aimed to investigate specific health parameters related to iron, resting metabolic rate and energy balance in a cohort of female athletes: those women who train and race on the world stage, yet do not have the (allied health) support staff that a professional athlete might access. This cohort may be at a higher risk of developing clinical health issues, such as iron deficiency, as demonstrated in the population studied in this thesis.

The first study, examined the validity of three pRMR equations against measured RMR and investigated the reliability of measured RMR using the TrueOne metabolic analyser (TrueOne 2400, Parvo Medics, Sandy UT). Although a small cohort, the Parvo Medics TrueOne analyser was determined to be a reliable tool for measuring RMR in recreationally-trained female athletes. Conversely, the validity of all three predictive equations when compared to measured RMR was questionable, with low levels of agreement. When grouped by training status, it was determined that the 3 equations underestimated RMR in the sub-elite female

athlete population by approximately 131-139 kcal·day⁻¹ but overestimated RMR in the recreationally-active females by approximately 148-162 kcal·day⁻¹. The predictive equation demonstrating the highest levels of agreement with measured RMR in both the recreationally-active and sub-elite female athletes was the Mifflin St. Jeor equation. As RMR is a tool used in the competitive sporting environment to determine energy availability and useful in the prevention of relative energy deficiency in sport (REDs); additional validation research in highly-trained female athletes is warranted.

As women are superseding men in endurance sport participation (da Fonseca-Engelhardt et al., 2013), a unique population is emerging that does not fit the definition of recreationally-active or elite. The second study of this thesis aimed to investigate specific health parameters in this unique population to determine iron status, resting metabolic rate, inflammation, and energy balance. Of the 13 women who were included in this study, six were classified as iron deficient (ID) and seven were classified as non-iron deficient (non-ID). Training hrs·wk⁻¹ showed a large correlation with measured RMR ($r = -0.66$, *large*) and intensity training (>80% VO_{2max}) hrs·wk⁻¹ also had a large correlation with BMD ($r = 0.68$, *large*). Results also suggested that clinical reference ranges for haematological measures may be inappropriate for this athletic population, as iron status should be higher for optimal performance. Moreover, this population of highly-trained, non-professional female athletes need additional support in understanding and diagnosing such issues as iron deficiency.

Practical applications

The outcomes of these studies aid in furthering the knowledge base surrounding female athlete health, more specifically in non-professional highly-trained female athletes. From the research conducted in the current studies, it was concluded that predictive RMR equations should be used with caution due to the risk of under-prediction of true energy availability in female athletes. Another health concern of this population is iron deficiency. This thesis demonstrates the prevalence of sub-optimal iron status in highly-trained female endurance athletes. Furthermore, the haematological profile of this population should be assessed with the knowledge that they are highly-active and optimal ranges for iron status may differ from the current clinical guidelines (Wians, 2018). As the cohort investigated in this thesis was small, further work is needed to validate and expand the knowledge base to inform health practices in highly-trained female athletes. The following recommendations are made for future research:

Future research

Measurement of RMR

- Research across a larger population of highly-trained athletes, both male and female, would be beneficial to further examine the validity of pRMR equations.
- There is also still limited knowledge on pre-test protocols. While most research suggests an overnight fast of at least 6-hours and no high intensity physically activity within 14-hours of testing (Compher et al., 2006), more

research in an athletic population may be beneficial, specifically addressing the effect that exercise has on RMR measurement as this is a large factor when testing athletes who are participating in set training regimes.

Health of highly-trained, non-professional female athletes

- Research on a more extensive population of highly-trained female athletes across a wide range of sports to expand the knowledge and literature base of this unique cohort would be beneficial. This can then be used to inform health practitioners and athletes alike of the unique risks afforded to them.
- There is still no clear set of guidelines for diagnosis of iron deficiency in athletes both female and male. The current standards applied to the female athlete are based upon a haemoglobin limit of 120 g/L (Van den Bosch et al., 2001), which is not applicable to the highly-trained female as training adaptations and the act of exercise itself, alters haematological parameters (Branch et al., 1997; Hu & Lin, 2012; Weight, Alexander, Elliot, & Jacobs, 1992).
- Oral contraceptives (OC) add complexity through the introduction of varying concentrations of circulating exogenous oestrogen and progesterone; which may moderate physiological adaptations to exercise differently than endogenous ovarian hormones, thus investigating and comparing health parameters of women on hormonal contraceptive to women who are naturally cycling should be considered.

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Appendices

Appendix 1 – Ethics approval

The University of Waikato
Private Bag 3105
Gate 1, Knighton Road
Hamilton, New Zealand

Human Research Ethics Committee & Human
Research Ethics Committee (Health)
Julie Barbour
Telephone: +64 7 837 9336
Email: humanethics@waikato.ac.nz



24th July 2017

Stacy Sims
Holly Thorpe
Kelsi McKay

Dear Stacy, Holly and Kelsi,

HREC(Health)#13 'A Transdisciplinary project on the physiological and socio-psychological health issues facing female athletes'

We have received your request to add a named student researcher, Kelsi McKay, to your project. Kelsi will be involved in data collection, and will use project data for the purpose of writing her Masters Thesis. The information to participants has been amended to reflect student involvement, and we are happy to approve this request.

Please contact the committee if you wish to make further changes to your project as it unfolds, quoting your application number HREC(Health)2017#13), with your future correspondence. Any minor changes or additions to the approved research activities can be handled outside the monthly application cycle.

Regards,

Julie Barbour PhD
Chairperson
University of Waikato Human Research Ethics Committee (Health)

Appendix 2 – Participant information

Document 1 – Survey information



SURVEY INFORMATION SHEET FOR FEMALE ATHLETE PARTICIPANTS

THE UNIVERSITY OF WAIKATO, Faculty of Education

A multidisciplinary project on the physiological health issues facing female athletes

Surveys

You will complete a short paper survey to gather:

1. Basic demographic information (~5 minutes) including:
 - i. Age
 - ii. Sport/event
 - iii. Years competing in your sport
 - iv. Highest achievements to date

Using MyFitness Pal:

2. Training information including:
 - i. Weekly training schedule
 - ii. Training duration and intensity
 - iii. Training modes
3. Standard dietary tracker to record energy intake across one (1) full week to include the weekend days (~30 minutes/day) including:
 - i. Meals and snacks of choice and frequency

- ii. Fluid consumption
- iii. Recovery nutrition
- iv. Menstrual Cycle Status

Confidentiality

As a participant in this project, you will remain anonymous in any materials produced from the research. Please let us know if you would be happy to be partially identified (e.g., age, sport) in our publications. See details regarding full confidentiality and partial disclosure on the informed consent form. The data collected will be used in research articles and presentations. Research articles will be published in academic journals and will be read mostly by university students, researchers, academics and sport and health professionals. The data will be presented to groups within universities and perhaps at academic conferences.

Participants' Rights

As a participant you have the right to:

- Discuss any concerns you have about the collection of the survey information
- Refuse to answer any particular question in the surveys
- Ask any further questions about the study which occur during your participation.
- Withdraw from the project at any time.
- Be given access to your survey information and to a summary of the findings from the study when it is concluded. Please provide a physical address or email address on the **Informed Consent Form** so that we can send you a copy of your results

Records

All records from the surveys will be kept confidential. They will be archived for at least five years according to University of Waikato Human Research Ethics Regulations, but it is expected they will be appropriately disposed of before 2022.

Document 2 – RMR and DXA information



RESTING METABOLIC RATE (RMR) ASSESSMENT and BODY COMPOSITION

INFORMATION SHEET FOR FEMALE ATHLETE PARTICIPANTS

THE UNIVERSITY OF WAIKATO, Faculty of Education

A multidisciplinary project on the physiological health issues facing female athletes

Resting Metabolic Rate (RMR) assessment (~30 minutes)

A RMR assessment will be conducted to establish your current energy availability status. For this assessment, you will be required to arrive at your chosen testing venue around 0730 following an overnight fast (nothing from 10pm the night before, except water). You will also be asked to refrain from exercising the day before.

Upon arrival, we will obtain your height and weight prior to the RMR assessment. You will be required to lie still and awake during the test. During this time, we will measure the amount of oxygen you consume and carbon dioxide you produce while lying down. To measure the expired gas, a ventilated hood will be placed over your head (Figure 1) for 15 minutes, which will be connected to a breath-by-breath gas analysis machine. You will be able to breathe freely throughout the entire test.



Figure 1. Example of a participant under a ventilated hood to measure resting metabolic rate (RMR)

Body Composition - Dual-energy X-ray absorptiometry (DXA) (~30 minutes)

If you have been identified with energy deficiency via the RMR assessment, you will be referred Bay Radiology to complete a DXA scan to assess your body composition. For this assessment, your fat mass, lean body mass, and bone health will be measured.

DXA provides the gold-standard assessment of body composition and is routinely used in Triad/RED-S related investigations to further detail energy deficiency status and bone health. It is non-invasive and painless. For this assessment, you will lay on your back on the DXA scanner bed for ~ seven minutes while the machine scans your body overhead from your head to your feet. The machine sends an invisible stream of low dose x-rays through your body via two energy streams. One is absorbed by soft tissues such as muscle, tendons and fat, the other by bone. Comfortable clothes should be worn, or a gown can be provided. You will be asked to remove any items that contain metal such as under wire bras, pants or shirts that have rivets or eyelets, belts, zips, buckles, and jewelry.

Body composition assessed using a DXA scan involves exposure to X-ray radiation. However, the amount of radiation used is very small. It is less than one tenth the dose of a standard chest x-ray. The risk will also be minimized by using trained professionals to carry out this assessment.

Confidentiality

As a participant in this project, you will remain anonymous in any materials produced from the research. Please let us know if you would be happy to be partially identified (e.g., age, sport) in our publications. See details regarding full confidentiality and partial disclosure on the informed consent form. The data collected will be used in research articles and presentations. Research articles will be published in academic journals and will be read mostly by university students, researchers, academics and sport and health professionals. The data will be presented to groups within universities and perhaps at academic conferences.

The results from your RMR (and DXA if referred) will be given only to you: it is up to you whom you decide to share this information with.

Participants' Rights

As a participant you have the right to:

- Discuss any concerns you have about the collection/storage/disposal of your results.
- Withdraw from the project at any time.
- Ask any further questions about the study which occur during your participation.
- Be given access to your physiological data and to a summary of the findings from the study when it is concluded. Please provide a physical address or email address on the Informed Consent Form so that we can send you a copy of your results.

Records

All records from the physiological testing will be kept confidential. They will be archived for at least five years according to University of Waikato Human Research Ethics Regulations, but it is expected they will be appropriately disposed of before 2022.

Document 3 – Blood sample information



BLOOD SAMPLE INFORMATION SHEET FOR FEMALE ATHLETE PARTICIPANTS

THE UNIVERSITY OF WAIKATO, Faculty of Education

A multidisciplinary project on the physiological health issues facing female athletes

Blood Samples (~20min)

Blood work will be important to identify key hormonal changes to identify whether you are at risk of RED-S symptoms.

Blood samples will be collected to determine your sex hormone profile, specifically cortisol, dehydroepiandrosterone (DHEA), and C-reactive protein (Crp). In female athletes with increased-elevated cortisol, there is a potential for menstrual cycle changes with the conversion of progesterone, estrogen, and testosterone to cortisol. Moreover, DHEA plays a role as it is involved in the synthesis of estrogen, progesterone, and testosterone in both men and women. Crp is a marker of inflammation which may indicate poor absorption of iron.

For this assessment, you will be seated in a sterile area of the lab prepared for a venepuncture of an arm vein. The insertion of a sterile needle, can sometimes cause discomfort, however a certified phlebotomist will do their best to prevent this. Once the needle is inserted into your vein, the extraction of blood is painless. Approximately 30 ml will be collected for this project (3x 10ml tubes).

When the blood has been collected, the needle will be removed, and pressure will be applied to the site to prevent further bleeding and bruising.

There are minimal risks associated with the collection of blood for this project via venepuncture which include:

1. Hematoma

2. Swelling, tenderness and inflammation at the site
3. Persistent bleeding
4. Vasovagal response – dizziness, sweating, coldness of skin, numbness and tingling of hands and feet, nausea, vomiting, possible visual disturbance, syncope (temporary loss of consciousness caused by a drop in blood pressure), and injury fall from fainting.

The risks are minimized by drawing minimal amount of blood for analysis, using a certified phlebotomist and following health and safety protocols. Special precautions to use sterile equipment and lab spaces, and to have a physician on call in case any adverse effects occur, will be implemented.

Analysis

All blood samples will be separated into four aliquots for analysis; one will be used immediately to measure hematocrit and hemoglobin, the rest will be transferred into tubes and spun to separate whole blood from serum. The serum will be separated and frozen for storage and transport, for analysis of sex hormones, cortisol, DHEA and Crp, later. The analyses will take place at PathLab, Tauranga. If you would like your samples returned, please let the research team know, otherwise, all samples will be destroyed (incineration) upon conclusion of the study.

Confidentiality

As a participant in this project, you will remain anonymous in any materials produced from the research. Please let us know if you would be happy to be partially identified (e.g., age, sport) in our publications. See details regarding full confidentiality and partial disclosure on the informed consent form. The data collected will be used in research articles and presentations. Research articles will be published in academic journals and will be read mostly by university students, researchers, academics and sport and health professionals. The data will be presented to groups within universities and perhaps at academic conferences.

The results from your blood work will be given only to you: it is up to you whom you decide to share this information with. If abnormal results are noted, you will be notified and referred to see your general practitioner or team doctor.

Participants' Rights

As a participant, you have the right to:

- Discuss any concerns you have about the collection/storage/disposal of blood samples.

- Refuse to answer any particular question
- Withdraw from the study at any time.
- Ask any further questions about the study which occur during your participation.
- Be given access to your physiological data and to a summary of the findings from the study when it is concluded. Please provide a physical address or email address on the Informed Consent Form so that we can send you a copy of your results.

Records

All records from the physiological testing and interviews will be kept confidential. They will be archived for at least five years according to University of Waikato Human Research Ethics Regulations, but it is expected they will be appropriately disposed of before 2022.

Appendix 3 – Informed consent



INFORMED CONSENT FORM

THE UNIVERSITY OF WAIKATO, Faculty of Education

A multidisciplinary project on the physiological health issues facing female athletes

I have read the **Information Sheet for Female Athlete Participants Documents** for this study and have had the details of the study explained to me. My questions about the study have been answered to my satisfaction and I understand that I may ask further questions at any time.

I agree to participate under the conditions set out below:

- 1) A member of the research team will conduct a resting metabolic rate assessment to establish current energy availability status. I, the participant, have the right to withdraw/withdraw data at any time. The referral for the DXA scan will only be completed if the research team identify you with energy deficiency.
- 2) A trained individual will conduct the collection of blood samples. I, the participant, have the right to withdraw/withdraw data at any time. The blood samples will be analysed in a clinical laboratory facility, and, if requested, samples will be returned to me; otherwise they will be destroyed upon the conclusion of the study.
- 3) I understand the research team will keep all records from the short paper surveys and physiological testing confidential. I understand that all data will be archived for at least five years according to University of Waikato Human Research Ethics Regulations but will most likely be appropriately disposed of before 2022.
- 4) The data collected by the research team will be used in research articles and presentations. I consent to the data being used for publication purposes. I understand that any use of the data will not take place without permission from me.

- 5) I understand the implications of choosing full confidentiality or partial disclosure, as conditions of confidentiality. *Full confidentiality* means that the researchers will not use my name or any other identifiers in anything they publish from this project. Choosing full confidentiality means they will identify me solely via the following: female, athlete, age range for the project. *Partial disclosure* means that the researchers will not use my name in anything they publish from this project, but I am happy for them to include my sporting code and/or specific age.
- 6) I understand that I can withdraw from this study at any time.
- 7) I understand that I can decline to answer any particular question in the study.
- 8) I understand that if I have any ethical concerns I can contact either the primary researchers, Dr Stacy Sims (email: stacy.sims@waikato.ac.nz) or Associate Professor Holly Thorpe (email: holly.thorpe@waikato.ac.nz) or Head of the University of Waikato Human Research Ethics Committee, Dr Julie Barbour (email: humanethics@waikato.ac.nz)

I agree to provide information for each assessment to the researcher under the conditions of confidentiality set out below:

Surveys	Full confidentiality []	Partial disclosure []
Resting Metabolic Rate	Full confidentiality []	Partial disclosure []
Body Composition (if referred)	Full confidentiality []	Partial disclosure []
Blood samples	Full confidentiality []	Partial disclosure []

Complete the relevant details below, adding pseudonym & other adapted information where required:

Signed:

.....

Date:

.....

Name (or pseudonym): Age:

Current contact details:

.....

☐ Please tick if you would like a copy of the project findings

Email:

.....